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Original Studies

Sample Size Determination for Evaluation of Time Domain Heart Rate Variability Indices in Canine Lameness

<rrh>Heart Rate Variability and Pain

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EKG (electrocardiogram); HRV (heart rate variability)

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ABSTRACT

Heart rate variability (HRV) is a physiologic phenomenon that occurs due to changing autonomic tone resulting in variable RR intervals. A reduction in HRV is used as an index of pain in neonatal human patients. Objective measures of pain would be valuable in the evaluation of canine patients and assessment of response to pain management strategies. We hypothesized that dogs with diseases associated with discomfort (osteoarthritis and bone neoplasia) would have reduced HRV compared with normal, healthy dogs. The aim of the study was to calculate the sample size necessary to investigate this hypothesis.

Seventeen dogs from the Ryan Veterinary Hospital of the University of Pennsylvania patient population or owned by Ryan Veterinary Hospital of the University of Pennsylvania staff were enrolled in this single-blind, prospective pilot study. A 30 min electrocardiogram (EKG) was obtained from each dog using an ambulatory electrocardiographic monitor. All EKGs were obtained between 10 a.m. and 2 p.m. EKGs were analyzed and time-domain HRV indices computed.

Sample size calculations suggest that 207 dogs would be necessary to ascertain if HRV is reduced in dogs experiencing discomfort or pain (50 in the arthritis group, 79 in the bone cancer group, and 78 in the control group).

<1>Introduction

Heart rate variability (HRV) is a physiologic phenomenon that occurs due to changing autonomic tone. It refers to the oscillation in the interval between consecutive R waves. HRV can be quantified by either time- or frequency-domain analysis. Time-domain analysis relies on mathematical quantification of the variability of RR intervals, whereas frequency-domain analysis is a more complex evaluation of the periodicity of RR intervals.[1]

Change in the HRV pattern is an early and sensitive indicator of compromised human health.[2] High HRV is a sign of a healthy individual whereas lower variability is an indicator of abnormal autonomic adaptability and the presence of physiologic abnormality.[2] Reduced HRV is a strong, independent predictor of mortality and arrhythmic complications after acute myocardial infarction, and reduced HRV has been consistently observed in human patients with cardiac failure.[3] It also predicts the clinical expression of autonomic neuropathy in diabetic patients.[3]

In humans, HRV is also a useful marker for many noncardiac diseases.[2] Reduced HRV has been used as a marker for pain in neonatal human patients and has been suggested as a method for measuring stress responses and welfare states in farm animal species.[4,5] The assessment of pain in canine patients is challenging, and few objective measures are available; such markers would have obvious clinical utility.

In dogs, the influence of a variety of cardiac diseases on HRV has been investigated.[6–8] However, HRV has not been evaluated in the presence of noncardiac disease, nor has its potential use as an objective marker of pain been investigated. The purpose of this pilot study was to determine the appropriate sample size necessary to ascertain if HRV is reduced in dogs experiencing discomfort or pain. Our hypothesis for this larger study is that time-domain indices of HRV would differentiate normal dogs from dogs experiencing lameness due to arthritis or bone cancer and that the magnitude of the decrease in HRV would be correlated with pain severity.

<1>Materials and Methods

This study was approved by the University of Pennsylvania Institutional Animal Care and Use Committee. Dogs with osteoarthritis (n = 6) or neoplasia (n = 4) causing lameness were evaluated at the Ryan Veterinary Hospital of the University of Pennsylvania by the Veterinary Clinical Investigations Center to be considered for enrollment in a pain management clinical

trial. At the time of this initial examination, no dogs were receiving therapy for pain management. As part of that study, a client questionnaire was completed regarding the perceived level of pain for each dog. Definitive diagnosis of the form of bone cancer was not necessary for enrollment in this study and was not available for most of the dogs. Clients who gave informed consent for their dog to be included in this pilot study evaluating HRV were asked to wait in a quiet room with their pet for the duration of the study. The dog was fitted with an ambulatory electrocardiographic monitor[a]: Sufficient fur was clipped over the areas of the left and right palpable apex heart beats and the skin wiped with acetone to remove surface grease. Once dry, adhesive electrocardiographic (EKG) electrodes were applied to the skin and the Holter monitor attached to the electrodes. A snugly fitting vest[b] was placed over the dog's thorax to prevent dislodgement; no bandage material was used. A period of acclimation was allowed. Once the dogs appeared relaxed, a 30 min EKG was obtained, during which time the dogs remained at rest in a small examination room with their owners. All EKGs were obtained between 10 a.m. and 2 p.m. to reduce the influence of circadian rhythms on the recordings. EKGs were uploaded into the Spacelab system using Impresario software. EKGs were manually analyzed beat by beat to ensure that beats were labeled correctly by the computer software. Time-domain HRV indices were then computed by the software, including successive NNs (normal RR intervals) differing by more than 50 ms, SDNN (ms) or the standard deviation of the NN interval, RMSDD (ms) or the square root of the mean squared differences of successive NN intervals, SDSD (ms) or the standard deviation of successive NN differences, TINN (ms) or the triangular interpolation of NN intervals, HRV index (ms), mean RR (ms), and mean heart rate (beats per min).
A group of seven normal dogs underwent the same EKG evaluation following owner consent. These animals were owned by hospital staff or students and were normal on physical examination, and no ectopy was present in their 30 min EKGs from which HRV was derived.

<2>Statistics

Statistical analysis was performed using commercially available software packages[c,d]. Data was assessed graphically for normality of distribution. For categorical data, groups were compared

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[a] Spacelabs Healthcare, Issaquah, Washington
[b] Surgi-Sox by DogLeggs, Reston, Virginia
[c] SPSS 22; IBM, Armonk, New York
[d] PSS; Vanderbilt University, Nashville, Tennessee

using Fisher exact test. Overall between-group comparisons were performed using Student *t* tests or Kruskall-Wallis tests, as appropriate.

<1>Results

Results are summarized in **Table 1**. There was no significant difference among groups for age ($P = .148$), sex ($P = .768$), or body weight ($P = .663$). There was no significant difference among groups for any of the time-domain HRV indices. No significant difference was detected in owner-estimated pain score between the osteoarthritis (mean = 15.8 ± 6.71) and neoplasia (mean = 19.3 ± 9.64) groups ($P = .714$). A sample size calculation suggested that a total of 207 dogs would be necessary to demonstrate a difference in time-domain HRV indices among groups (50 in the arthritis group, 79 in the bone cancer group, and 78 in the control group).

<1>Discussion

HRV analysis has been shown in several studies to measure autonomic nervous system activity, which is strongly influenced by pain or stress.[2,3] Results from various human studies evaluating subjects ranging from pain in neonates to patients with posttraumatic stress disorder have suggested that decreased HRV may be a useful indicator of pain.[4,9] However, to our knowledge, this is the first study to evaluate HRV as an indicator of pain in clinical canine patients.

Our results suggest that changes in time-domain indices of HRV are relatively small in situations of stress or discomfort in dogs, and therefore relatively large numbers of dogs would be necessary to determine whether significant differences exist. Factors other than pain may have contributed to the findings of the present study. For example, all of the measurements were performed in the hospital, and it is possible that the stress of being in the hospital masked any effect of pain or discomfort on HRV. Evaluation of HRV in dogs within their home environments would be necessary to further investigate this possibility. It is also possible that discomfort is not associated with measureable changes in HRV indices in dogs, such that an appropriately powered study would not demonstrate significant differences among groups. Further study using the calculated sample size of 207 dogs is therefore warranted to further investigate the possible utility of time-domain indices of HRV as markers of stress or discomfort in dogs.

In the present study, 30 min recordings were obtained between 10 a.m. and 2 p.m. This 4 hr time window was selected to allow the owners of the dogs some flexibility in scheduling the time of their participation, while reducing the influence of circadian rhythms on the results of the study. In the morning, sympathetic tone is dominant, whereas in the afternoon, parasympathetic tone is dominant.[2] Previous studies have not identified an optimal data acquisition period, with investigators reporting periods of between 5 min and 24 hr. However, it is believed that reliable data is best obtained under controlled conditions, and so a 30 min acquisition period during rest with the owners present was chosen to minimize variability.[2]

<1>Conclusion

In conclusion, the results of this pilot study suggest that a total of 207 dogs would be necessary to ascertain if HRV is reduced in dogs experiencing discomfort or pain (50 in the arthritis group, 79 in the bone cancer group, and 78 in the control group).

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TABLE 1

Population Data and Indices of Time-Domain Heart Rate Variability for Arthritis, Neoplasia, and Control Groups

Characteristics, *n* = 17	**Arthritis, *n* = 6**	**Neoplasia, *n* = 4**	**Control, *n* = 7**	***P***
Age, yr (range)	8.5 (8.0–10.3)	9.5 (6.0–13.0)	8.0 (7.0–8.0)	.148
Weight, kg (range)	32.5 (24.8–43.8)	54.5 (26.0–71.8)	31.0 (28.0–33.7)	.663
Sex, male/female	2/4	2/2	2/5	.768
Successive NNs, number differing by >50 ms (range)	753.5 (538.0–853.5)	1510.0 (453.5–1740.0)	1041.0 (326.0–1571.0)	.372
SDNN, ms (range)	91.2 (83.3–119.4)	193.1 (70.9–344.2)	143.8 (84.4–250.5)	.272
RMSDD, ms (range)	64.5 (58.7–97.2)	277.1 (58.2–414.7)	166.8 (52.9–198.9)	.240
SDSD, ms (range)	52.3 (44.6–80.4)	161.4 (42.2–268.0)	140.6 (43.9–171.4)	.254
HRV index, ms (range)	12.5 (10.4–13.6)	15.1 (7.9–18.8)	12.2 (8.0–15.7)	.876
TINN, ms (range)	332.1 (140.7–375.0)	242.2 (175.8–384.8)	296.9 (156.3–437.5)	.945
Mean RR, ms (range)	527.9 (467.9–629.0)	776.4 (530.0–951.5)	643.9 (512.1–833.1)	.178
Mean HR, bpm (range)	117.5 (99.0–135.5)	85.1 (70.9–116.7)	98.2 (78.2–120.2)	.179

The median and interquartile ranges are shown for continuous variables. *P* is the probability that no difference exists between groups.

HR, heart rate; HRV, heart rate variability; NN, normal RR intervals; SDNN, standard deviation of the NN interval; RMSDD, square root of the mean squared differences of successive NN intervals; SDSD, standard deviation of successive NN differences; TINN, triangular interpolation of NN intervals.